

# Meeting Minutes

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Date | Time | 5/17/2016 10:00 AM |  
Pacific Standard Time

Location | The Lands Council (TLC)  
Saranac Building  
25 West Main Ave, Spokane  
2<sup>nd</sup> Floor Large Conference Room

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Project | Fungi PCB Research

Conference Line | Ex. 6 Personal Privacy (PP) Pin# Ex. 6 Personal Privacy (PP)

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## Reference Documents:

- VW Experimental Design Detailed 05112016
- VW Experiment Equipment List
- Proposed VW Characterization
- VW Disposal Methods

## Attendees in person:

Doug Greenlund, City of Spokane  
Mike Petersen, The Lands Council  
Heidi Montez, The Lands Council  
Aimee Navickis-Brasch, NBSWE  
Greg Lahti, WSDOT  
Jeff Donovan, RPWRF  
David Cleary, Gonzaga

## Attendees on conference line:

Alex Taylor, Fungi Perfecti  
Michelle Mullin, EPA Region 10  
Mark Maurer, Thurston County  
Maureen Johnson, Certified Safety Professional

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## Meeting Minutes

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The meeting opened with the attendees introducing themselves.

Heidi provided an overview of updates to experimental design and phase 1 timeline since the last meeting (defined on the attachments sent out with the agenda).

The following are comments discussed during the meeting:

### Vactor Waste Sample Collection

The Vactor Waste will be collected using the procedures defined in the Cities QAPP: *PCB characterization of Spokane Regional Vactor Waste Decant Facilities*.

- **Action item:** Jeff Donovan will coordinate with the lab and provide the containers and any specifics regarding storage and shipping samples.
- **Action Item:** Aimee will add Vactor Waste Collection procedures to project QAPP
- **Action Item:** Vactor Waste Sample Collection planned for June 2<sup>nd</sup> and the collection team is: Heidi Montez, Aimee Navickis-Brasch, Greg Lahti

### Sterilizing and Mixing Vector Waste with Agar

2 options were discussed during the meeting:

1. Mix VW into Agar, pour together
    - Method: autoclave jar of VW 3 times in city's lab, add to agar and pressure cook together, pour dishes
    - Would be easier than adding to each dish
    - Is this more of a health concern?
    - Will the VW sink to bottom of dish?
  2. Add VW to cooled agar dishes
    - More labor intensive
    - VW will sit on surface of agar
- Michelle Mullin expressed concerns about the potential for dioxins being formed during *pressure cooking/autoclaving Vector Waste*. (Sterilization in pressure cooker: 250 degrees F, 15 PSI). She recommended adding an analysis of the sterilized material to look for dioxins and/or dioxin-like congeners (12). (formation of dioxins at temperatures between 450-750 F under 1 atmosphere). Michelle also suggested we investigate the vapor pressure of PCBs at 250F and 2 atm to determine if they will volatilize and potentially become airborne.
  - Lisa Rodenburg responded to the group regarding this concern in an email dated 5/18/16. She indicated that we shouldn't be worried about the formation of dioxins. She also mentioned that measuring dioxins is more difficult, and as expensive as measuring PCBs. She attached a chart of PCB congeners and their corresponding vapor pressures and indicated volatilization under those conditions is unlikely.
  - **Action items:** select one of the 2 sterilization methods and determine if Heidi needs to wear a respirator/ take other precautions during this process.

### Characterization and Detection of PCBs

David Cleary mentioned there may be an affordable, cheap initial screening method, to detect changes in PCB levels. **Rapid Sediment Characterization of PCBs With ELISA** (Information about this method was sent to the group after the meeting).

- Limitations of Method:
  - Semi-quantitative, non-specific data (cannot speciate between different Aroclor TM mixtures or individual congeners).
  - Must know site condition and contaminants.
  - Field screening data are not always accepted for regulatory compliance, but can be used to develop sampling strategies.
  - Cross-reactivity for some classes of contaminants.
  - Tests require stringent attention to kit's procedure.
- **Action item:** Ask the City if this is a method they would like to evaluate and compare to the other testing that is already being conducted for this study. Adrian Borgess (Ecology) may also have some insight into using this method for detecting changes in PCB concentrations.

- **Action Item:** Doug is investigating whether the city lab can assist with homogenizing and determining the gradation of vector waste.

Alex asked about the PCB concentrations from this one sample and how we would verify they are representative of typical concentrations.

- **Action Item:** When the PCB results are back, Aimee will compare with concentrations from other testing conducted at both the WSDOT and City decant facilities and provide a short summary about the representativeness of the samples.

Alex recommended adding metals testing (Cu, Zn) to Vector Waste characterization because Zn can be toxic to fungi and affect growth. Testing methods recommended by the group include: PCB congeners, TPH, Nitrate-Nitrite, Phosphorus, Particle distribution, pH, zinc, copper, Chlordane, DDT, bulk density, and moisture content.

- **Action item:** Aimee will determine cost of additional testing. Also, Aimee and Heidi will go over proposed characterization list with the city and determine if additional testing methods will be added to this study. (*attached characterization list has been updated by Jeff Donovan*).
- **Action Item:** Heidi will ask Philip if he has any other suggestions for the parameters to be characterized.

### Experimental Procedures

Alex Taylor provided comments regarding training fungi to Vector Waste: The concept of 'training' or 'acclimating' the fungus to a particular food source allows the fungus to get used to the food, 'build a tolerance' or 'acquire a taste' for it. Alex is concerned that the current timeline of this step of the experiment (growing each fungus on one VW petri dish) is too short. He explained that this process requires multiple generations (multiple transfers of the fungus to another Vector Waste petri dish), because you are essentially 'selectively breeding' the fungus. When the fungus makes contact with the new food source (vector waste) it may start expressing genes that it wasn't previously using. For example, the fungus might start producing new enzymes for digesting that food source (VW), that it wasn't producing before. You can transfer this fungus (and its VW eating enzymes) to another VW petri dish, and let it grow and produce more VW eating enzymes. The idea is to encourage the fungus' ability to digest the VW by introducing it again and again to more VW petri dishes. The concept is that the fungus essentially 'evolves' to tolerate the food you are giving to it. Alex feels the current timeline for this step is too short. He said this isn't going to happen on a single dish, you need multiple generations of selecting good growth and transferring it. Also, some species grow much more slowly than others, so the transferring schedule will become staggered. (Coincidentally, this will spread out the work for each step, which will make it easier to manage.) Alex also mentioned that we should expect contamination (for example, mold) on the petri dishes, even while using sterile technique. Any time you start adding foreign substances (like VW), contamination becomes harder to avoid. He suggests doing at least twice as many replicates as in the current design (6 VW petri dishes per species instead of only 3)

- **Action Item:** Heidi will investigate the growth rate of each fungus to determine how this might impact the project timeline. (*Attached is a growth parameter chart including growth rates on sawdust and incubation temp. no data on growth rates on agar dishes.*) Then she will create two timelines; 1) Unchanged/original (may require removing fungi from the study), and 2) Revised schedule to accommodate extended 'training' time (growth rates)
  - Per discussions at a meeting on May 25, the city has agreed to extend the research project timeline beyond the current 12/31/16 date. The Lands Councils is developing a revised research schedule and budget that will account for the extended timeline (primarily Heidi's salary) and adding the following items: a final report summarizing the study findings and two presentations by The Lands Council to Council Public Works Committee (PWC) (July 2016 and at the end of the project).

Expect contamination; add more replicates (6-9 VW dishes per species instead of 3)

- consider using a surfactant ("tween" biofriendly soap) may help improve solubility of extracellular enzymes; a potential idea for later studies

Heidi has some questions on the equipment list and asked the group for recommendations for selecting these items.

- **Action Item:** Alex is going to provide Heidi feedback on the equipment list.

#### Sawdust Source/ Quality

Surewood Custom Cabinets is currently collecting alder or maple (both will work) sawdust for the project (need 15 gallons); particle size unknown. The group indicated that a coarse size would be optimum for this study because it allows for air space between particles. If this is not the optimum size (maybe too small or too big) will need to find another source. Fungi Perfecti sells alder saw dust (\$10 per 10lb bag). The group discussed this option however it was recommended that we find a material that is readily available in the area. Other options suggested include: Poplar Tree Mill in Lewiston ID or Borman Oregon; Other Mills in Hayden, ID, or stove pellets

- **Action Item:** Heidi will determine the gradation (size) of the Surewood sawdust ASAP. If the material is too small, investigate other potential sources.

The group recommend that in addition to the volume to volume ratios for Task 5, dry weight to dry weight ratios should be added (because bulk density can vary). An estimate on the moisture content is as follows:

- Analytical moisture balance-
- Hydrated sawdust= 60-65% moisture content
- Grain spawn= 40% moisture content
- **Action Item:** We need to determine the moisture content of the materials as well as the, gradation, bulk density (we may need to specify the density at a specific compaction). Heidi will ask Mark and Alex for recommendations on methods.